



Institute Report No. 355

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Acute Dermal Toxicity of Nitrosoguanidine in Rabbits

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and
Don W. Korte, Jr., PhD, LTC, MSC

MAMMALIAN TOXICOLOGY BRANCH
DIVISION OF TOXICOLOGY

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for William P. Corby 11 Sept 89
Donald G. Corby (date)
COL, MC
Commanding

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ABSTRACT

The acute dermal toxicity of nitrosoguanidine, was evaluated in five male and five female New Zealand White rabbits. Nitrosoguanidine (2 g/kg) was applied topically to the clipped dorsal skin surface under a semi-occlusive wrap for 24 hours. There was no evidence of percutaneous systemic toxicity or death. Nine of the rabbits exhibited slight to moderate erythema after wrap removal and all had cleared by 24 hours. Slight erythema was also observed in 3 rabbits 48-72 hours after wrap removal. These data indicate that nitrosoguanidine does not produce systemic toxicity when administered by 24-hour topical application at a limit dose of 2 g/kg.

KEY WORDS: Acute Dermal Toxicity, Nitrosoguanidine, Rabbit, Mammalian Toxicology, Nitroguanidine

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PREFACE

TYPE REPORT: Acute Dermal Toxicity GLP Report

TESTING FACILITY:

US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129-6800

SPONSOR:

US Army Medical Research and Development Command
US Army Biomedical Research and Development Laboratory
Fort Detrick, MD 21701-5010
Project Officer: Gunda Reddy, PhD

PROJECT/WORK UNIT/APC: 3E162720A835/180/TLB0

GLP STUDY NUMBER: 85011

STUDY DIRECTOR: Don W. Korte, Jr., PhD, LTC, MSC
Diplomate, American Board of Toxicology

PRINCIPAL INVESTIGATOR: Earl W. Morgan, DVM, MAJ, VC, Diplomate
American College of Veterinary Preventive
Medicine, American Board of Toxicology

CO-PRINCIPAL INVESTIGATOR: SSG James D. Justus, MPA

PATHOLOGIST: G. Tracy Makovec, DVM, MAJ, VC, Diplomate
American College of Veterinary Pathologists

REPORT AND DATA MANAGEMENT:

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCE: Nitrosoguanidine

INCLUSIVE STUDY DATES: 10 October 1985 - 12 November 1985

OBJECTIVE:

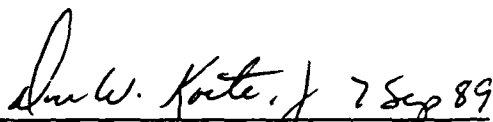
The objective of this study was to evaluate the acute dermal toxicity of nitrosoguanidine in male and female New Zealand White rabbits.

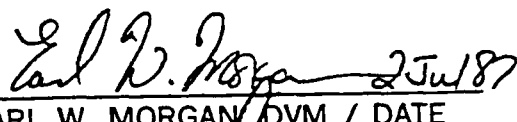
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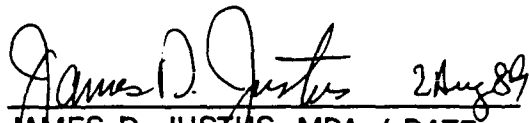
LTC Larry D. Brown, DVM, SP4 James J. Fischer, SP4 Theresa L. Polk, and SP4 Scott L. Schwebe assisted in conducting this research; Diane Arevalo, Obie B. Goodrich, and Richard A. Spieler provided care for the animals; SGT Paul B. Simboli, BS, assisted in the chemical analysis; and Colleen S. Kamiyama and Julie Peacock provided secretarial assistance.

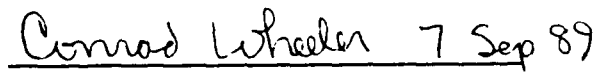
SIGNATURES OF PRINCIPAL SCIENTISTS INVOLVED IN THE STUDY

We, the undersigned, declare that GLP Study 85011 was performed under our supervision, according to the procedures described herein, and that this report is an accurate record of the results obtained.


DON W. KORTE, JR., PhD / DATE
LTC, MSC
Study Director


EARL W. MORGAN, DVM / DATE
MAJ, VC
Principal Investigator


JAMES D. JUSTUS, MPA / DATE
SSG, USA
Co-Principal Investigator


CONRAD R. WHEELER, PhD / DATE
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Analytical Chemist



DEPARTMENT OF THE ARMY
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REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

8 September 1989

MEMORANDUM FOR RECORD

SUBJECT: GLP Compliance for GLP Study 85011

1. This is to certify that the protocol for LAIR GLP Study 85011 was reviewed on 10 May 1985.
2. The institute report entitled "Acute Oral Toxicity of Nitrosoguanidine in Rabbits," Toxicology Series 171, was audited on 6 September 1989.

Carolyn M. Lewis

CAROLYN M. LEWIS, MS
Diplomate, American Board of
Toxicology
Quality Assurance Auditor

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Acute Dermal Toxicity of Nitrosoguanidine in Rabbits—Morgan *et al.*

INTRODUCTION

Nitrosoguanidine is a potential anaerobic degradation product of nitroguanidine (1), a primary component of US Army triple-base propellants, which is now produced in a Government-owned contractor-operated ammunition plant. The US Army Biomedical Research and Development Laboratory (USABRDL), as part of its mission to evaluate the environmental and health hazards of military-unique propellants generated by US Army munitions-manufacturing facilities, conducted a review of the nitroguanidine data base and identified significant gaps in the toxicity data (2). The Division of Toxicology, LAIR, was tasked by USABRDL to develop a genetic and mammalian toxicity profile for nitroguanidine, related intermediates/by-products of its manufacture, and its environmental degradation products.

Objective of Study

The objective of this study was to determine the acute dermal toxicity of nitrosoguanidine in male and female New Zealand White rabbits.

MATERIALS

Test Substance

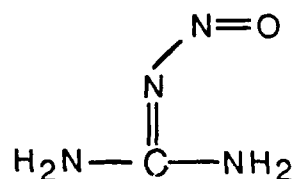
Chemical Name: Nitrosoguanidine

Chemical Abstracts Service Registry No.: 647-81-7

LAIR Code Number: TP48

Physical State: Yellow powder

Chemical Structure:



Molecular Formula: CH₄N₄O

Other test substance information is presented in Appendix A.

Vehicle

To enhance its penetration into the skin, nitrosoguanidine was moistened with 0.9% saline at the time of application. Saline was obtained from Tavenol Laboratories, Inc. (Deerfield, IL). The lot number was 3C979X6 and the expiration date was June 1986.

Animal Data

Five male and five female young New Zealand White rabbits (Elkhorn Rabbitry, Watsonville, CA) from a shipment that arrived at LAIR on 10 October 1985 were assigned to the study. One rabbit (85F231) in the shipment was submitted for necropsy quality control on 11 October 1985. The 10 rabbits were identified individually by ear tattoos. The animal weights ranged from 2.1 to 2.7 kg on receipt and from 2.6 to 2.9 kg at dosing. Additional animal data appear in Appendix B.

Husbandry

The rabbits were housed individually in stainless steel wire mesh cages in racks equipped with automatic flushing dumptanks. No bedding was used in any of the cages. Water was provided *ad libitum* by continuous drip from a central line. The diet consisted of approximately 150 g per day of Purina Certified Rabbit Chow[®] No. 5322 (Ralston Purina Company, St. Louis, MO). The animal room temperature was maintained at 18.3 to 21.7°C and the

relative humidity was maintained at 51% to 73%, except for minor fluctuations due to room cleaning. The photoperiod was 12 hours of light per day.

METHODS

This study was performed in accordance with LAIR Standard Operating Procedure OP-STX-30, "Acute Dermal Toxicity Study" (3) and Environmental Protection Agency guidelines (4).

Acclimation/Group Assignment

Study rabbits were quarantined by the Division of Animal Care and Services, LAIR, for two weeks before being certified healthy by a staff veterinarian. During quarantine the rabbits were given ketamine (100 mg *im*) for ear tattooing during which time one application of Canex®/mineral oil (Pitman-Moore, Inc., Washington Crossing, NJ) was applied for ear mite protection. After being certified healthy, the rabbits were transferred to the Toxicology Suite for the remainder of the study.

Randomization for group assignment was unnecessary as there was only one dose level for each sex.

Dose Levels

A "limit test" was conducted in which 5 male and 5 female rabbits were assigned to a test group receiving 2.0 g/kg of nitrosoguanidine applied topically to the dorsum (skin over back). According to body weight, 5.1 to 5.8 g of nitrosoguanidine was applied to each rabbit.

Compound Preparation

The calculated amount of nitrosoguanidine was mixed with up to 5 ml of 0.9% saline to form a paste. This paste was prepared immediately before applying it to the animal.

Chemical Analysis of Nitrosoguanidine

Previous testing had indicated that nitrosoguanidine was stable in an aqueous vehicle for a period exceeding the time needed to prepare and apply the paste to the rabbits' backs (Appendix A).

Test Procedures

The application sites on the dorsal and lateral sections of the animals (surface area approximately 300 cm²) were close-clipped with electric clippers (Oster® Model A5, Size 40 blade, Sunbeam Corp, Milwaukee, WI) 24 hours before applying the test compound. The animals were weighed, and the quantity of compound required to provide the 2.0 g/kg limit dose was measured. This quantity of the test compound was evenly distributed over the surface of a 7 x 7 in. piece of gauze dressing (Curity Cover Sponges, Kendall Co. Hospital Products, Boston, MA) which was then taped to the animal's back with hypoallergenic tape (Durapore® Surgical Tape, 3M Corp, St. Paul, MN). The trunk of the animal was then wrapped with Vetrap® bandaging tape (Animal Care Products, 3M Corp, St. Paul, MN) to hold the compound in place and prevent the animal from ingesting the compound. The Vetrap® was anchored in place cranially and caudally by strips of Conform® elastic tape (Kendall Co. Hospital Products, Boston, MA). The patch and wrappings were left in place for 24 hours. No restraint of the animals was used except during the wrapping procedure. When the wrappings and patch were removed, the exposed area was gently wiped with a piece of saline-moistened gauze to remove any remaining test compound.

Observations

Observations for mortality and signs of acute toxicity were performed daily according to the following procedure: (1) animals were observed undisturbed in their cages, (2) animals were removed from their cages and given a physical examination, and (3) animals were observed after being returned to their cages. On the day of dosing, the animals were checked intermittently throughout the day. Observations were recorded daily for the

remainder of the two-week test period. A second "walk-through" observation was performed each day, with only significant observations recorded. The exposed area was examined daily after patch removal for signs of dermal reaction. Animals were weighed weekly during the study test period.

During evaluation of the exposure site, area and intensity of each dermal reaction were graded. Grading was performed according to a scale which included five categories to describe area and severity. Area categories were 0 - 5%, > 5 - 10%, > 10 - 25%, > 25 - 50% and > 50%; severity was defined as slight, mild, moderate, and severe.

Necropsy

All study animals were submitted for necropsy. Those that survived the 14-day study period were necropsied immediately after being given an overdose of sodium pentobarbital and sacrificed by exsanguination from severed axillary vessels. Skin was taken from the exposed area and examined microscopically.

Duration of Study

The study period was 14 days and was preceded by a 19-day quarantine. Historical study events are listed in Appendix C.

Changes/Deviations from Protocol

The hygrothermograph used in the animal room was miscalibrated on 21 October. The humidity readings during the period 21-24 October read between 70% and 75%. The hygrothermograph was also miscalibrated on 4 November and the reading for that day was about 73%. The proper readings based on readings before and after recalibration for this period should have been approximately 58%. There was a steam outage in the building on 26 November with a resulting fluctuation in the temperature and humidity readings during the day. The animal care technician on duty did not record clinical observations on 6 November. None of these changes appeared to have any effect on the study.

Raw Data and Final Report Storage

A copy of the final report, study protocols, raw data, retired SOPs, and an aliquot of the test compound will be retained in the LAIR Archives.

RESULTS

Twenty-four hour dermal exposure to nitrosoguanidine at a limit dose of 2.0 g/kg produced no mortality in the 10 rabbits evaluated in the study. During the course of the study, observations were split into two major categories: systemic (general health of the animal) and dermal.

Systemic: Three rabbits (85F237, 85F238, 85F239) had fecal material matted in the perianal areas at 2, 4, and 24 hours after dosing. Rabbit 85F239 was also inactive at 2 and 4 hours after dosing and rabbit 85F235 did not eat during the first 24 hours and was inactive 48 hours after dosing. None of the clinical systemic signs were interpreted as signs of toxicity attributable to nitrosoguanidine. The rabbits gained weight, as expected for young animals, during quarantine and after administration of nitrosoguanidine (Appendix D).

Dermal: Skin irritation signs are presented in Appendix E. Erythema was observed in 9 of 10 rabbits 1/2 hour after patch removal. By 24 hours after patch removal the erythema had disappeared in all animals. Slight erythema was observed in three rabbits at 48 hours and persisted through 72 hours.

There were no gross or microscopic findings in these rabbits at necropsy, following the 2-week observation period, that could be attributed to dermal exposure to nitrosoguanidine at the 2 g/kg dose level. A copy of the Pathology Report appears in Appendix F.

DISCUSSION

Acute dermal toxicity testing is designed to evaluate both systemic toxicity due to percutaneous absorption of the test material and local toxicity from its contact with the skin. From these observations it can be determined whether absorption of the test material across the skin is sufficient to produce systemic effects or lethality. In the present study, nitrosoguanidine produced slight local dermal reactions with no evidence of systemic effects.

All of the animals exposed to a limit dose of 2.0 g/kg nitrosoguanidine survived to the end of the test. None of these test animals exhibited any clinical signs suggestive of a systemic action by nitrosoguanidine. This lack of acute dermal toxicity is in marked contrast with acute intraperitoneal administration of nitrosoguanidine, which was lethal at doses as low as 21 mg/kg in mice (5). Therefore, it is concluded that dermal exposure to nitrosoguanidine, at 2.0 g/kg, either does not result in sufficient percutaneous absorption to produce systemic toxicity or is not a systemic toxin at doses tested in the rabbit. The dermal median lethal dose of nitrosoguanidine, as indicated by this study, is above the limit value of 2.0 g/kg.

Local dermal toxicity was observed at the site of exposure. As summarized in Appendix E, slight to moderate erythema was present in 9 of 10 animals after the removal of test compound wrappings. Erythema is a relative nonspecific reaction to a dermal insult; however, the recurrence of erythema in these rabbits may suggest a more specific response to the test compound.

CONCLUSION

A limit dose of 2.0 g/kg nitrosoguanidine was not lethal to rabbits nor did it produce significant systemic effects following dermal exposure for 24 hours.

REFERENCES

1. Kaplen DL, Cornell JH, Kaplen AM. Decomposition of nitroguanidine. *Environ Sci Technol* 1982; 16: 488-492.
2. Kenyon KF. A data base assessment of environmental fate aspects of nitroguanidine. Frederick, MD: US Army Medical Bioengineering Research and Development Laboratory, 1982, DTIC No ADA 125591.
3. Acute dermal toxicity study. LAIR Standard Operating Procedure OP-STX-30, Presidio of San Francisco, CA: Letterman Army Institute of Research, 18 May 1984.
4. Environmental Protection Agency. Office of Pesticides and Toxic Substances, Office of Toxic Substances (TS-792). Acute exposure, dermal toxicity. In: Health effects test guidelines. Washington, DC: Environmental Protection Agency, August 1982; EPA 560/6-82-001.
5. Epstein SS, Arnold E, Andrea J, Bass W, Bishop Y. Detection of chemical mutagens by the dominant lethal assay in the mouse. *Toxicol Appl Pharmacol* 1972; 23:288-325.

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Appendix A: CHEMICAL DATA

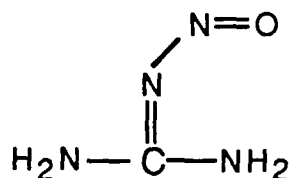
Chemical Name: Nitrosoguanidine

Chemical Abstracts Service Registry No.: 674-81-7

Lot Number: WCC-2-002

LAIR Code: TP48

Chemical Structure:



Molecular Formula: CH₄N₄O

Molecular Weight: 88

Physical State: Yellow powder

Analytical Data:

HPLC: Nitrosoguanidine was analyzed using conditions similar to those employed by Burrows *et al.*¹ Conditions were as follows: column, Brownlee RP-18 (4.6 mm x 25 cm); mobile phase, water; flowrate, 0.8 ml/min. The effluent was monitored at 255 nm. The retention times for nitrosoguanidine and nitroguanidine were 4.4 and 6 min, respectively. The HPLC data demonstrated that the nitrosoguanidine contained approximately 2.5% nitroguanidine.²

IR (KBr): 3378, 3096, 1690, 1649, 1508, 1341, 1266, 1134, 1088, 1035, 690, 668 cm⁻¹.³

Solubility:

A saturated solution of nitrosoguanidine in water was prepared at room temperature. A 1:500 dilution of this solution produced an absorbance of 0.533 units. Using an extinction coefficient of 13,305 L/moles-cm, the concentration of nitrosoguanidine in the original saturated solution was calculated to be 1.76 mg/ml.⁴

Appendix A(cont.): CHEMICAL DATA

Stability:

Stable for at least 4 hours in water for at least 4 hours at room temperature.⁵

Source: Alan Rosencrance

US Army Biomedical Research and Development Laboratory
Fort Detrick, Maryland

¹ Burrows EP, Brueggeman EE, Hoke SH. Chromatographic trace analysis of guanidine, substituted guanidines and striazines in water. *Chromatog* 1984;16:494-8.

² Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 37. Letterman Army Institute of Research, Presidio of San Francisco, CA.

³ *Ibid.* p 30.

⁴ Wheeler CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #85-01-006, p 66. Letterman Army Institute of Research, Presidio of San Francisco, CA.

⁵ Wheeler, CR. Nitrocellulose-Nitroguanidine Projects. Laboratory Notebook #84-05-010.3, p 32-36. Letterman Army Institute of Research, Presidio of San Francisco, CA.

Appendix B: ANIMAL DATA

Species: *Oryctolagus cuniculus*

Strain: New Zealand White (albino)

Source: Elkhorn Rabbitry
5265 Starr Way
Watsonville, CA 95076

Sex: Male and female

Age: Young adult Date of Birth: 29 Aug 85 - Males
25 Aug 85 - Females

Animals in each group: 5 males and 5 females

Condition of animals at start of study: Normal

Body weight range at dosing: 2.6 - 2.9 kg

Identification procedures: Ear tattoo.

Pretest conditioning:

1. Quarantine/Acclimation from 10 - 28 October 1985
2. Animals were close-clipped and examined 24 hours before dosing

Justification:

The laboratory rabbit is a proven mammalian model for dermal toxicity studies because of its size, ease of restraint, and skin permeability.

Appendix C: HISTORICAL LISTING OF STUDY EVENTS

<u>DATE</u>	<u>EVENT</u>
10 Oct 85	Animals arrived at LAIR. They were observed for illness and held for a two-week quarantine period.
10 Oct - 12 Nov 85	Animals were observed daily.
11,18 Oct 85	Animals were weighed.
18 Oct 85	Animals were tattooed.
24 Oct 85	Animals were removed from quarantine, transferred to the GLP Suite, and weighed.
28 Oct 85	Animals were close-clipped.
29 Oct 85	Animals were weighed, dosed, and observed for clinical signs.
30 Oct - 12 Nov 85	Animals were observed daily for clinical and dermal signs.
5,12 Nov 85	Animals were weighed.
12 Nov 85	Feed was removed during the morning observation. Animals were submitted to the Necropsy Suite.

Appendix D: BODY WEIGHT DATA

Animal Number	Day					
	<u>01</u>	<u>08</u>	<u>014</u>	<u>0</u>	<u>7</u>	<u>14</u>
<u>Females</u>						
85F230	2500*	2605	2774	2826	2530	3234
85F232	2465	2520	2720	2626	2340	2873
85F233	2330	2460	2585	2720	2946	3116
85F234	2710	2830	2890	2927	3050	3202
85F235	2230	2485	2585	2643	2520	2931
Mean	2447.0	2580.0	2710.8	2748.4	2677.2	3071.2
Standard Deviation	182.4	150.1	130.2	127.3	304.7	161.7
<u>Males</u>						
85F236	2140	2345	2503	2620	2810	3003
85F237	2170	2430	2509	2576	2730	2811
85F238	2180	2495	2624	2657	2276	3081
85F239	2210	2580	2719	2754	2538	3119
85F240	2475	2675	2786	2860	3060	3283
Mean	2235.0	2505.0	2628.2	2693.4	2682.8	3059.4
Standard Deviation	136.5	128.3	125.5	113.9	294.6	172.4

* Weights are given in grams.

Appendix E: INDIVIDUAL DERMAL SIGNS

<u>Animal Number</u>	<u>Dermal Signs</u>	<u>Duration of Dermal Signs(Days)</u>	<u>Severity*</u>	<u>Area†</u>
Females				
85F230	Erythema	1	A	2
85F232	Erythema	1	A	3
85F233	Erythema	1	A	3
85F234	Erythema	1-4	A-B	4
85F235	Erythema	1	A	1
Males				
85F236	Erythema	1-4	A-C	5
85F237 *	Erythema	1	A	3
85F238	Erythema	1(day 3)	A	1
85F239<	Erythema	1	A	3
85F240	Erythema	1	A	2

* Severity Scores

A = Slight
 B = Mild
 C = Moderate
 D = Severe

† Pertains to percent of exposed area exhibiting signs of dermal irritation.
 This value is determined by visual approximation.

1 = 5%
 2 = > 5 to 10%
 3 = >10 to 25%
 4 = >25 to 50%
 5 = >50%

< Moderate molting was observed on 10-25% of the application site from Days 1-7.

Appendix F: PATHOLOGY REPORT

Acute Dermal Toxicity Study
Nitrosoguanidine
GLP Study 85011

Study: GLP #85011, Toxicology Services Group

Test substance: Nitrosoguanidine.

Species: Rabbit, New Zealand White.

Method of euthanasia: Sodium Pentobarbital (IP).

Investigator: CPT Earl Morgan.

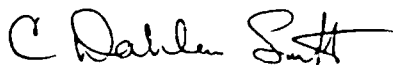
History: These animals arrived at LAIR on 10 October 1985 as part of a shipment of 27 animals from Elkhorn Rabbitry, Watsonville, California. They underwent two weeks of quarantine in ARG and were dosed topically with Nitrosoguanidine (2 gm/kg) on 29 October 1985. The wrap was left on for 24 hours in accordance with OP-STX-30, dated 18 April 1984.

Gross Necropsy Findings:

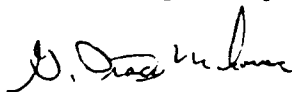
LAIR ACC#	ANIMAL ID#	SEX	MICROSCOPIC EXAM	MICROSCOPIC FINDINGS
38443	85F230	F	YES	Not remarkable (NR)
38444	85F232	F	YES	NR
38445	85F233	F	NO	NR
38446	85F234	F	NO	NR
38447	85F235	F	NO	NR
38448	85F236	M	YES	NR
38449	85F237	M	NO	NR
38450	85F238	M	NO	NR
38451	85F239	M	NO	NR
38452	85F240	M	YES	NR

Gross Findings: No lesions were recognized except for Animal #85F237, which had a 1cm abscess located on the left side of the prepuce. In accordance with the protocol, the skin of 2 animals of each sex was examined. One section of skin was taken from the area of exposure over the mid-dorsal back. A similar control sample was taken from the skin over the sacrum.

Comment: The compound produced no gross or microscopic changes.



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29 January 1986

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